Association of EGFR expression level and cetuximab activity in patient-derived xenograft models of human non-small cell lung cancer

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Abstract

Purpose: To explore in a panel of patient-derived xenograft models of human non-small cell lung cancer (NSCLC) whether high EGFR expression, was associated with cetuximab activity.

Experimental Design: NSCLC patient-derived xenograft models (n=45) were implanted subcutaneously into panels of nude mice and randomization cohorts were treated with either cetuximab, cisplatin, cisplatin plus cetuximab, vehicle control, or else were left untreated. Responses according to treatment were assessed at week 3 by analyzing the relative change in tumor volume and an experimental analogue of the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines. An EGFR immunohistochemistry score was calculated for each patient-derived xenograft model and response was assessed according to EGFR expression level.

Results: When tumors were stratified into high and low EGFR expression groups (immunohistochemistry score threshold 200; scale 0–300), a stronger antitumor activity was seen in the high EGFR expression group compared with the low EGFR expression group in both the cetuximab monotherapy and cisplatin plus cetuximab combination therapy settings. For tumors treated with cisplatin plus cetuximab, the objective response rate was significantly higher in the high EGFR expression group compared with the low EGFR expression group (68% vs 29%). Objective response rates were similar in high and low expression groups for tumors treated with cisplatin alone (27% vs 24%, respectively).

Conclusion: Cetuximab activity in NSCLC patient-derived xenograft models was demonstrated clearly only in tumors that expressed high levels of EGFR, as defined by an immunohistochemistry score of ≥200.
Translational relevance

The phase III FLEX study in patients with NSCLC showed that the survival benefit associated with the addition of cetuximab to chemotherapy was limited to patients whose tumors expressed high levels of EGFR, as defined by an immunohistochemistry score of ≥200 (scale of 0–300). This study investigated in an extensive panel of patient-derived xenograft models of human NSCLC whether cetuximab activity was similarly associated with high tumor EGFR expression when cetuximab was administered alone or in combination with cisplatin. Objective responses to cetuximab monotherapy were seen only in tumors with EGFR expression levels determined to be ≥200. Furthermore, the addition of cetuximab to cisplatin was associated with a significant improvement in response rate in tumors with high EGFR expression but had no apparent effect in tumors with low EGFR expression. This provides further evidence that in the treatment of advanced NSCLC, high tumor EGFR expression can predict cetuximab benefit.
Introduction

The efficacy of a particular anticancer drug may be associated with the presence or absence of specific genetic driver lesions within the cells of a tumor. Such lesions may be point mutations, chromosomal translocations or increases in gene copy number (1-6). Perhaps related to such changes in the genome, the level of expression of the target protein of an anticancer drug within tumor cells can also represent a predictive biomarker on which treatment decisions may be based (7, 8). The discovery and clinical application of predictive tumor biomarkers is a key enabling factor in the emerging field of personalized medicine, which allows the selection of subgroups of patients most likely to benefit from treatment with a particular anticancer drug.

The addition of the EGFR antibody, cetuximab, to platinum-based chemotherapy was shown to improve response rate and overall survival compared with chemotherapy alone in the first-line treatment of patients with advanced non-small cell lung cancer (NSCLC) (9). However, in the absence of a predictive biomarker the effect was modest in an unselected patient population. The randomized phase III trial FLEX comparing the combination of cetuximab plus cisplatin and vinorelbine vs cisplatin and vinorelbine was the largest study in this setting (10). An analysis of prospectively collected tumor EGFR immunohistochemistry data from FLEX study patients explored whether EGFR expression level was predictive for cetuximab activity (11). This led to the identification of a discriminatory threshold immunohistochemistry score of 200 on a scale of 0–300 which allowed the separation of a patient subgroup benefiting from the addition of cetuximab to chemotherapy (high EGFR expression; immunohistochemistry score ≥200) from one deriving little or no benefit (low EGFR expression; immunohistochemistry score <200). For patients in the high EGFR expression group (n=345, 31%), overall survival was longer in the chemotherapy plus cetuximab arm compared with the chemotherapy alone arm (median 12.0 months vs 9.6 months; hazard
ratio 0.73; \( P=0.011 \) and the response rate was higher (44.4\% vs 28.1\%; odds ratio 2.04; \( P=0.002 \)). No corresponding benefit for the addition of cetuximab to chemotherapy was seen for patients in the low EGFR expression group. Treatment interaction tests assessing the difference in the hazard ratios for overall survival and odds ratios for response suggested a predictive value for EGFR expression level based on the threshold immunohistochemistry score of 200.

Recently, a similar finding was reported from the RTOG 0617 trial exploring the addition of cetuximab to radiotherapy and standard chemotherapy for the treatment of patients with unresectable stage III NSCLC. While adding cetuximab to chemoradiotherapy did not improve overall survival in an unselected patient population, patients with tumors showing high EGFR expression level according to a hybrid immunohistochemistry score were significantly more likely to benefit from the addition of cetuximab than patients with low EGFR expression (12).

It has been suggested that patient-derived xenograft model systems may more faithfully represent the characteristics of primary human tumors than xenografts of established human tumor cell lines (13-16). The aim of the current study was to investigate whether high tumor EGFR expression levels, as defined in the FLEX clinical study by an immunohistochemistry score of \( \geq 200 \), were associated with response to therapy in an extensive panel of patient-derived NSCLC xenografts grown in athymic nude mice when cetuximab was administered alone or in combination with cisplatin. The intention was therefore to further explore in an experimental setting the biological rationale that high EGFR expression may be a predictive biomarker for cetuximab treatment benefit in NSCLC.
Materials and Methods

Patient-derived NSCLC xenografts and mouse strains

All experiments were carried out on behalf of Merck KGaA by one of three contract research organizations: Oncotest, (Freiburg, Germany), Experimental Pharmacology & Oncology (Berlin, Germany) and Xentech (Evry, France).

The patient-derived xenograft models were established from surgically resected tumor specimens from patients with NSCLC (see Supplementary Table S1) by subsequent serial passaging in immune-deficient mice. The passage numbers varied from passage 5 to 33 (see Supplementary Table S1). The following host mice strains were used: Female NMRI nu/nu (Experimental Pharmacology & Oncology and Oncotest), female athymic nu/nu (Xentech). The xenografts from Experimental Pharmacology & Oncology were described previously (17). All experiments were conducted in accordance with relevant local regulations and guidelines relating to animal welfare.

The only inclusion criterion of a particular patient-derived NSCLC model in the study was the availability of the model during a certain time period at the respective contract research organization. Consequently, the xenografts used in this analysis represent a broad diversity of human NSCLC characteristics and include models derived from primary and metastatic sites and those representing a variety of different histological subtypes (see Supplementary Table S1).

Experimental design

Each NSCLC xenograft assessed (n=45) was surgically excised from its murine host and cut into 50 fragments of approximately 2–5 mm diameter. These fragments were then implanted subcutaneously using tweezers into the flanks of 50 anaesthetized nude mice (or fewer depending on availability) via small incisions (one tumor fragment per animal). Animals were subsequently monitored daily and when transplanted tumors became palpable or reached a
volume of approximately 80–200 mm³ they were randomized to five experimental groups of up to 10 mice. Three of the five groups received anticancer drug treatment comprising either cetuximab 30 mg/kg intraperitoneal (i.p.) injection twice weekly, cisplatin 5 mg/kg i.p. injection once weekly or cisplatin combined with cetuximab, at the monotherapy dose levels. On days on which both cisplatin and cetuximab were administered, cetuximab was given one hour prior to cisplatin. Mice in the control group received i.p. injections of the cisplatin/cetuximab vehicle (0.9% NaCl) according to the schedule for cetuximab. Response was assessed after 3 weeks of treatment. Animals randomized to the fifth group (biomarker arm) received no treatment and tumors were harvested at a size of 300–400 mm³.

Body weights were recorded twice a week. Tumor volume was determined using two-dimensional measurements taken with a caliper on the same day that mice were weighed. Mice were sacrificed if the volume of their tumor reached 2000 mm³.

Data availability and patient derived tumor models

Forty-five NSCLC patient-derived xenograft models were included in the data analysis. EGFR immunohistochemistry data were obtained from 41 tumors. A complete data set (efficacy data and immunohistochemistry data) was not available for all tumors, leading to a reduced number evaluable for certain analyses. Tumors for which EGFR expression data were not available were only considered in analyses relating exclusively to the differential effects of therapy. In addition, as certain tumors grew relatively quickly, mice in some instances had to be sacrificed before week 3. Thus, the number of patient-derived xenograft models contributing to the presented data varies between 37 and 45 depending on the analysis.
**EGFR expression analysis**

Where possible, for each human NSCLC patient-derived xenograft model, EGFR expression was assessed by immunohistochemistry in tumors from 5 of 10 randomly selected untreated mice from the biomarker arm. Tumors were sampled when they reached a volume of 300–400 mm$^3$, formalin-fixed and embedded in paraffin. They were subsequently analyzed at a central pathology laboratory using the EGFR pharmDx™ kit (Dako, Glostrup, Denmark) according to a standard protocol, without knowledge of treatment outcome. A median EGFR immunohistochemistry score on a scale of 0–300 was calculated from all tumors analyzed per patient-derived xenograft model based on the intensity and frequency of membrane staining of tumor cells, as previously described (11). This score was used to classify the NSCLC patient-derived xenograft models into high (immunohistochemistry score $\geq$200) or low (immunohistochemistry score <200) EGFR expression groups.

**Analysis of EGFR kinase domain and KRAS mutations**

The *EGFR* kinase domain mutation status of the patient-derived xenografts was assessed in two, randomly selected tumors from the biomarker arm. DNA was extracted with the Qiagen QIAamp DNA FFPE Tissue kit according to the manufacturer's instructions and quantified using a Nano Drop spectrophotometer. Detection of *EGFR* mutations was carried out using the CE-IVD marked Qiagen Therascreen EGFR PCR kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol.

*KRAS* mutation status of tumors was assessed in frame of a CAGE (Cancer Genome) scanner analysis performed by Blackfield, Cologne, Germany. Per patient-derived xenograft one randomly selected tumor from the biomarker arm was analyzed. DNA was extracted from 10 sections (each 10 $\mu$m) derived from fresh frozen tumor material using the Qiagen Gentra Puregene Kit according to the manufacturer's protocol. DNA concentrations were measured using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies, Darmstadt, Germany). The principle of CAGE is based on the hybrid capture technology (18). Baits, targeting the genomic regions of interest, were designed based on an up-to-date
list of cancer relevant genes, observed to be mutated, fused, amplified or deleted in cancer using Agilent SureDesign. Oligonucleotide composition was adapted based on locus specific features of the region of interest. Input genomic DNA was sheared and subjected to library preparation according to the manufacturer’s protocol. The baits and genomic DNA libraries were mixed to enrich for the genomic regions of interest followed by in-depth sequencing. Sequencing was performed by ATLAS Biolabs GmbH, Berlin, Germany.

Statistical methods and considerations

Study endpoints included the ratio of relative tumor volumes (compared with treatment start) in cetuximab treated compared with control tumors and cisplatin plus cetuximab treated compared with cisplatin treated tumors (treatment/control, T/C, ratio). Objective response at week 3 was also assessed according to an experimental analogue of the Response Evaluation Criteria in Solid Tumors (RECIST) score (19).

T/C ratios, defined as the ratio of the relative median tumor volume for both treatment arms at time t and start of treatment, were calculated at week 3 according to the formula:

\[
\frac{T}{C} = \frac{\text{median}_{d=41\ldots410}[T_{t}]}{\text{median}_{d=41\ldots420}[C_{t}]} \times \frac{\text{median}_{d=41\ldots420}[C_{\text{start}}]}{\text{median}_{d=41\ldots410}[T_{\text{start}}]}
\]

Where data (animals A1–A10) were available for less than 10 animals per group, endpoint calculations were based on as many tumors as were evaluable. T/C ratios <1 (represented graphically as log2 values <0) indicated tumor growth inhibition in the treatment arm compared with the control arm. A NSCLC xenograft was dropped from the analysis if endpoint data were missing entirely for one experimental arm.
Objective response was determined according to a RECIST score calculated from the percentage change in median relative tumor volume at week 3 and the median tumor volume at the start of treatment (defined as 100%) according to the formula:

$$RECIST\%_{X,w3,\text{av}} = \text{median} \left( \frac{V_{X,w3,AV}}{V_{X,\text{start},AV}} \times 100 \right) - 100$$

Objective response was defined as complete (RECIST score -100%) or partial remission (≤-30% to >-100%). Stable disease was defined by a RECIST score of ≤20% to >-30%, and progressive disease by scores of >20%.

A number of different statistical tests and regression models were used in the assessment of treatment effects and associations, including Wilcoxon signed-rank test, Pearson correlation, Mann-Whitney test, Fisher’s exact test, and mixed-effects logistic regression model. Tests were applied according to which endpoints, which EGFR expression categorization and which treatment arms were to be evaluated. Significance was assigned to test results that passed a $P$-value threshold of <0.05. Statistical tests were carried out as implemented in the statistics software R (http://cran.r-project.org).

A key objective of the study was to assess the effect of treatment with cetuximab or cisplatin plus cetuximab according to whether tumors expressed high (immunohistochemistry score ≥200) or low (immunohistochemistry score <200) levels of EGFR. Outcome was therefore assessed both in high vs low EGFR expression groups as well as according to EGFR immunohistochemistry score considered as a continuous variable. Where the EGFR immunohistochemistry score was not available for a xenograft, tumors were excluded from all expression level-related analyses.
Response rates according to EGFR immunohistochemistry score for cetuximab monotherapy compared with control and cisplatin plus cetuximab compared with cisplatin alone were assessed using the subpopulation treatment effect pattern plot (STEPP) method (20). In a sliding window approach, (tumors ordered by EGFR immunohistochemistry score; window size of a 25%-quartile range of scores) objective response rates and median immunohistochemistry scores were determined for each window and plotted accordingly.

Results

Patient-derived xenograft bearing animals were randomized to the 4 different experimental groups: cetuximab, cisplatin, cisplatin plus cetuximab, and vehicle control. In addition, animals were allocated to a biomarker group which did not receive any treatment and which was used for molecular analysis. Treatments started when tumors were well established, at volumes of approximately ~80 to 200 mm³.

Cetuximab monotherapy

For the determination of efficacy using T/C ratios for cetuximab monotherapy versus vehicle control, 42 tumors were evaluable (Figure 1A). Cetuximab administration resulted in a significant reduction of tumor growth in this broad panel of patient-derived xenograft models, demonstrated by T/C ratios <1 for most models (P=2.95 x 10⁻⁷, non-parametric one-sample Wilcoxon signed-rank test). When applying the RECIST model for the evaluation of response to cetuximab, an objective response was observed in seven of 42 patient-derived xenografts, corresponding to a response rate of 17%.

The antitumor activity of cetuximab (indicated by low T/C ratios) was particularly pronounced in tumors with high EGFR expression levels (Figure 1B), which was reflected by a significant
correlation of log₂ T/C ratio at week 3 and EGFR immunohistochemistry score (Pearson correlation coefficient r=-0.39; \( P=0.016 \)). When an EGFR immunohistochemistry score threshold of 200 (the same as identified in the FLEX study) was used to stratify the NSCLC xenografts into high (immunohistochemistry score \( \geq 200 \); n=21) and low (immunohistochemistry score <200; n=16) expression groups, strong responses to cetuximab versus control were exclusively observed in the group with high EGFR expression. This was illustrated by significantly different log₂ T/C ratios at week 3 between the two groups (\( P=0.0013 \), Mann-Whitney test; Figure 1C).

In line with these results, objective responses to cetuximab treatment measured according to the RECIST-analogous endpoint were seen only in tumors with EGFR immunohistochemistry scores \( \geq 200 \) (n=7; \( P=0.0437 \), Mann-Whitney test; Figure 2A and B). This corresponded to a response rate of 32% in the high EGFR expression group versus 0% in the low EGFR expression group (7/22, 32% vs 0/16, 0%; \( P=0.0144 \), Fisher’s exact test). Not all tumors with high EGFR expression showed sensitivity to cetuximab treatment, indicating that additional factors determine the response to anti-EGFR therapy.

**Cisplatin plus cetuximab versus cisplatin alone**

The phase III FLEX study compared cisplatin-based chemotherapy plus cetuximab with chemotherapy alone. Therefore, in the current study we assessed the effect of adding cetuximab to cisplatin, with T/C ratios calculated using the cisplatin group as the comparator. T/C ratios for cisplatin plus cetuximab versus cisplatin treatment could be calculated for 45 xenograft models (Figure 3A). The addition of cetuximab to cisplatin led to an overall improvement in the inhibition of tumor growth compared with cisplatin alone (\( P=0.00012 \), non-parametric one-sample Wilcoxon signed-rank test).

Again, when looking at the association of EGFR expression level with tumor volume in the cisplatin plus cetuximab treatment group and corresponding cisplatin treatment group, a
trend for better outcome (lower log₂ T/C ratios) at week 3 was apparent in tumors with higher EGFR expression levels (Pearson correlation coefficient $r=-0.26$; $P=0.12$; Figure 3B). When evaluable tumors were stratified according to the threshold immunohistochemistry score of 200, log₂ T/C ratios were lower ($P=0.0131$, Mann-Whitney test) in the high EGFR expression group ($n=22$) compared with the low EGFR expression group ($n=17$), consistent with a more pronounced inhibition of tumor growth from cisplatin plus cetuximab compared with cisplatin alone in the high EGFR expression group (Figure 3C).

Adding cetuximab to cisplatin doubled the overall response rate, measured as objective response according to the RECIST model, from 26% (10/39) for cisplatin alone to 51% (20/39) for cisplatin plus cetuximab. This increase in response rate was seen predominantly in the group with high EGFR expression levels (Figure 4). In tumors with high EGFR expression the addition of cetuximab to cisplatin markedly increased the response rate from 27% (6/22) to 68% (15/22). In tumors with low EGFR expression there was no marked difference in response rates between the two treatment arms in the group (cisplatin: 4/17, 24%; cetuximab plus cisplatin: 5/17, 29%). The association of high EGFR expression with increased response rate was clearly confined to the cisplatin plus cetuximab arm (5/17 vs 15/22; $P=0.0248$, Fisher’s exact test). In the cisplatin alone arm, there was no significant difference in response rate between the low and high EGFR expression groups (4/17 vs 6/22; $P=1.00$, Fisher’s exact test).

The increase in response rate associated with the addition of cetuximab to cisplatin in NSCLC patient-derived xenograft models with high EGFR expression was most likely due to more than one mechanism. Certain tumors appeared to be highly sensitive to anti-EGFR therapy alone, with the cetuximab treatment making the main contribution to the observed antitumor activity. However, in other tumors, neither cetuximab nor cisplatin alone resulted in the best antitumor activity, but the combination of both agents was needed to achieve the
maximal response. Examples for both phenomena are provided in Figures 5A and 5B, respectively.

**Impact of EGFR and KRAS mutation status**

None of the tumors was found to harbor an EGFR kinase domain mutation. In 11 of 41 analyzed tumors (27%) a KRAS mutation was detected (see Supplementary Table S1). KRAS mutations were present in tumors with high and low EGFR expression. No association between efficacy of cetuximab (monotherapy or in combination with cisplatin) and KRAS mutation status was found (data not shown). This is in line with clinical results from phase III studies indicating that KRAS mutation status is not a predictive biomarker for cetuximab efficacy in NSCLC (21, 22). The predictive value of EGFR expression for the efficacy of cetuximab in NSCLC appeared to be independent of the KRAS mutation status.

**STEPP analysis of response according to immunohistochemistry score**

To further explore whether an EGFR immunohistochemistry score of 200 was an effective threshold for predicting the activity of cetuximab in the present study, thereby confirming the threshold determined in the FLEX study, response rates according to treatment group were estimated for different EGFR immunohistochemistry scores in sliding windows according to a STEPP approach. For cetuximab monotherapy compared with control (no active treatment), EGFR immunohistochemistry scores \( \geq 200 \) (Figure 6A) were clearly associated with responses to therapy. In the case of cisplatin plus cetuximab compared with cisplatin alone, the STEPP plot confirmed that a threshold of 200 would effectively differentiate a subgroup of tumors likely to derive the most benefit from the addition of cetuximab to chemotherapy (EGFR immunohistochemistry score \( \geq 200 \)) from one deriving little or no benefit (Figure 6B).

Further confirmation that an EGFR immunohistochemistry score of 200 was an effective threshold in relation to cetuximab activity was provided by an analysis investigating the difference in log2 T/C ratios when the tumors were grouped according to a range of scores.
from 150, increasing in increments of 10, to 250 (Supplementary Table S2). For cetuximab monotherapy compared with control as well as for cisplatin plus cetuximab compared with cisplatin alone, the most significant T/C differences were seen with threshold scores of 200 and 210. This provides further evidence that a discriminatory EGFR threshold of 200 is effective in relation to the identification of tumors most likely to respond to cetuximab monotherapy.

Discussion

Using immunohistochemistry and response data to select an outcome-based discriminatory immunohistochemistry score threshold, the FLEX study demonstrated that high tumor EGFR expression could be used to define a subgroup of patients with advanced NSCLC who would derive a meaningful survival benefit from the addition of cetuximab to first-line cisplatin and vinorelbine chemotherapy (11). This observation is in line with the mode of action of cetuximab, which is thought to depend on direct interaction with the EGFR molecule (23).

The current study was designed to explore whether the association between high EGFR expression, as defined in the FLEX analysis by an immunohistochemistry score of ≥200 on a scale of 0–300, and cetuximab activity could also be demonstrated in vivo in an extensive panel of patient-derived NSCLC xenografts categorized for EGFR expression level. Indeed, this proved to be the case, with objective responses to cetuximab monotherapy seen only in tumors with EGFR expression levels determined to be ≥200. The addition of cetuximab to cisplatin was associated with a marked improvement in response rate in tumors with high EGFR expression (EGFR immunohistochemistry score ≥200) but had no apparent effect in those tumors with low EGFR expression (EGFR immunohistochemistry score <200). Interestingly, some patient-derived xenografts with high EGFR expression did not show
strong sensitivity to cetuximab or cisplatin alone but did show a strong response when the agents were administered in combination. Response rates in the cisplatin alone treatment groups appeared to be independent of EGFR expression level, being similar in the low and high EGFR expression subgroups.

The in vivo data relating to response from the current analysis are therefore consistent with the response and survival data from the FLEX study in indicating that an EGFR immunohistochemistry score of ≥200 can predict a treatment benefit in NSCLC associated with the addition of cetuximab to chemotherapy.

In contrast to these clinical and experimental findings in NSCLC, EGFR expression level as determined by immunohistochemistry does not appear to be of predictive value in relation to the treatment benefit associated with the addition of cetuximab to chemotherapy regimens used in the first-line treatment of metastatic colorectal cancer or recurrent and/or metastatic squamous cell carcinoma of the head and neck (24). In these two settings, the addition of cetuximab to chemotherapy appeared to improve survival and PFS essentially across the full EGFR immunohistochemistry score range, with no evidence for a clinically meaningful threshold EGFR immunohistochemistry score that might identify subgroups of patients with and without benefit. In metastatic colorectal cancer it has been clearly demonstrated that the KRAS mutational status is a predictive biomarker for cetuximab, with cetuximab benefit limited to those patients who have KRAS wild-type tumors (3, 25, 26). In contrast, in the first-line treatment of advanced NSCLC, KRAS mutational status does not appear to be of predictive value in patients receiving chemotherapy plus cetuximab (21, 22). In line with these observations, no association between the KRAS mutational status and cetuximab efficacy was seen in this study. Thus, while patients with KRAS wild-type metastatic colorectal cancer can derive benefit from cetuximab treatment independently of tumor EGFR expression levels, it is the EGFR expression level that appears to predict cetuximab efficacy in NSCLC, not the KRAS mutation status. These findings provide further evidence that
individual biomarkers, which may be predictive for outcome in relation to a particular targeted agent in one tumor type or setting, may not be equally informative for that agent in other tumor types or settings.

To our knowledge, this is one of the first studies where the predictive value of a biomarker has been demonstrated in a clinical phase III trial and also independently, in a large panel of patient-derived xenograft models of the same disease. This is of special interest given the increasing number of studies suggesting the usefulness of patient-derived tumor models in the drug discovery process. In particular, by directly transplanting human tumors into murine hosts, the histopathological features, tumor heterogeneity, genetic profiles and gene expression patterns can be preserved over several passages of the patient-derived xenograft models in mice (13-16, 27). Interestingly, also the expression of proteins crucial for cancer development like MEK1/2 and p44/p42 MAPK remains stable over different passages as shown by a recent study investigating uveal melanoma patient-derived xenograft models (28). Nevertheless, in the current study tumors from the biomarker arm coming from the same passage as the treated tumors were used for the determination of biomarkers to prevent any potential bias due to differences in passage number. It has been suggested that patient-derived xenograft models might be able to predict more faithfully the chemoresponsiveness of tumors than xenografts initiated from cancer cell lines (17). While early studies showed the value of these models in relation to mirroring the response patterns of primary tumors to individual chemotherapeutic agents (17, 29), more recently, a strong correlation has been demonstrated between drug activity in patient-derived xenograft models and clinical antitumor responses to those same drugs in the originally biopsied patients (30). However, a study investigating whether the predictive value of a molecular biomarker identified in a large phase III study can be confirmed in an extensive panel of patient derived xenografts has not yet been reported. While many xenografts used in this study were derived from patients with early stage tumors a substantial number was derived from metastasis (see Supplementary Table S1). The majority of the original tumors were treatment naïve. No patient was pretreated with cetuximab. Thus, at least to some extent...
the original tumors used for the generation of xenografts in this study can be expected to resemble the tumors of patients enrolled in the FLEX study. Of note, responses to cetuximab monotherapy and to cetuximab plus cisplatin in this study were seen in high EGFR expressing patient-derived xenografts classified as adenocarcinoma, squamous cell carcinoma and pleomorphic carcinoma (see Supplementary Table S1). This is also in line with observation made in the FLEX study indicating that the predictive effect of EGFR expression levels for cetuximab activity appeared to be independent of the histological subtype (11).

The close agreement in this case between the in vivo models and the FLEX study EGFR expression analysis further supports the notion that patient-derived xenograft models might be an effective way to increase the predictive utility of animal tumor models and suggests that they might be used to prioritize candidate biomarkers for testing in the clinical setting.

A final confirmation of the predictive value of high EGFR expression for the efficacy of cetuximab in advanced NSCLC has to come from further clinical studies. Just recently the SWOG trial S0819 (NCT00946712) added measurement of an EGFR immunohistochemistry score and its association with PFS and overall survival as an additional secondary objective. With an estimated enrollment of 1546 patients, the trial compares overall survival in patients with advanced NSCLC treated with carboplatin, paclitaxel and bevacizumab, with vs without cetuximab (31). Although the setting is different due to the presence of bevacizumab, the analysis might help to collect further clinical evidence for EGFR expression as a predictive biomarker for cetuximab in NSCLC.

In conclusion, analysis of a panel of patient-derived xenograft models of NSCLC suggested that cetuximab activity was limited to tumors that expressed high levels of EGFR, as defined by an immunohistochemistry score of ≥200 on a scale of 0–300. This was the case when cetuximab was administered as monotherapy, or when it was administered in combination research.
with cisplatin chemotherapy. The experimental data are therefore consistent with the previously reported biomarker analysis of the phase III FLEX study which indicated that high EGFR expression (as defined by an immunohistochemistry score ≥200) may be an effective predictive biomarker for distinguishing patients with NSCLC who are likely to benefit from the addition of cetuximab to platinum-based chemotherapy from those unlikely to benefit (immunohistochemistry score <200).

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Figure legends

**Figure 1.** A, T/C ratios (independent of EGFR expression level) across the NSCLC xenograft panel for cetuximab monotherapy/control. Tumors are ordered by log₂ of the T/C ratio. Value of 0 indicates no effect of cetuximab administration on tumor volume. B, T/C ratios for cetuximab monotherapy/control according to EGFR expression level on a continuous scale. C, T/C ratios for cetuximab monotherapy/control according to EGFR expression level in low and high EGFR expression groups (upper/lower boundaries of each box plot represent the 25th and 75th percentile; horizontal line within the box denotes the median value).

**Figure 2.** EGFR immunohistochemistry score and objective response in the cetuximab monotherapy arm. A, Tumor EGFR immunohistochemistry score range according to response category (for derivation of box plot parameters, see Figure 1). B, response rate in low and high EGFR expression groups.

**Figure 3.** A, T/C ratios (independent of EGFR expression level) across the NSCLC xenograft panel for cisplatin plus cetuximab/cisplatin. B, T/C ratios for cisplatin plus cetuximab/cisplatin treatment according to EGFR expression level on a continuous scale. C, T/C ratios for cisplatin plus cetuximab/cisplatin treatment according to EGFR expression level in low and high EGFR expression groups (for derivation of box plot parameters, see Figure 1).

**Figure 4.** Objective response to cisplatin or cisplatin plus cetuximab treatment in the low and high EGFR expression groups.

**Figure 5.** Antitumor response to cetuximab, cisplatin and the combination of cisplatin plus cetuximab in two patient-derived NSCLC xenograft models: A, in xenograft IC1TEP
(immunohistochemistry score, 280), the objective response in the cisplatin plus cetuximab group is driven by a high sensitivity to cetuximab; B, in xenograft LXFA 586 (immunohistochemistry score, 210), only the combination of cisplatin plus cetuximab results in an objective response.

SEM, standard error of the mean.

**Figure 6.** STEPP analysis of objective response rate by immunohistochemistry score for cetuximab monotherapy compared with no active treatment (A) and cisplatin plus cetuximab compared with cisplatin alone (B). Response rates for each treatment group were calculated in sliding windows across the range of the EGFR immunohistochemistry score. Each window contained response data from 25% of the analyzed tumors plotted against the median immunohistochemistry score.

OR, objective response.
References


Figure 1.

B

![Graph showing log T/C (week 3, cetuximab/control) vs. EGFR IHC score. Pearson $r = -0.39$, $P = 0.016$.]

C

![Graph showing log T/C (week 3, cetuximab/control) for EGFR IHC score <200 and ≥200. Mann-Whitney test, $P = 0.0013$.]
Figure 2.

A

B

Mann-Whitney test

P=0.0437

Response rate (%)

EGFR IHC score <200

EGFR IHC score ≥200

32%

0%
Figure 3.

A

Wilcoxon signed rank test

\[ P=0.00012 \]
Figure 4.

Response rate (%) for different treatment regimens and EGFR IHC scores:
- Cisplatin: 24%
- Cisplatin + cetuximab: 29% for EGFR IHC score <200 and 68% for EGFR IHC score ≥200.
Figure 5.

A days of treatment
tumor volume [mm$^3$]
mean ± SEM

- Vehicle
- Cetuximab, 30 mg/kg, 2x/week
- Cisplatin, 5 mg/kg, 1x/week
- Cetuximab + Cisplatin

Research.
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B

Days of treatment
Tumor volume [mm$^3$] mean ± SEM

0 2 4 6 8 10 12 14 16 18 20 22

Vehicle
Cetuximab, 30 mg/kg, 2x/week
Cisplatin, 5 mg/kg, 1x/week
Cetuximab + Cisplatin

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Figure 6.

A
Cetuximab vs untreated controls: response rates for tumors in intervals of EGFR IHC scores

B
Cisplatin + cetuximab vs cisplatin: response rates for tumors in intervals of EGFR IHC scores

Objective response rate in IHC interval (%) vs EGFR IHC score.
Association of EGFR expression level and cetuximab activity in patient-derived xenograft models of human non-small cell lung cancer

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